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INHIBITION OF POLYRIBOINOSINIC:POLYRIBOCYTIDYLIC ACID-INDUCED DEPRESSION OF MOUSE HEPATIC MIXED FUNCTION OXIDASES BY ACTINOMYCIN D OR CYCLOHEXIMIDE

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Summary. Administration of a single dose of the potent interferon inducer poly rI:rC to Swiss Webster mice depressed hepatic cytochrome P-450 to 75% of control, ethylmorphine N-demethylase to 56% of control and DMN N-demethylases I and II to about 80% of control. Although each enzyme responded in a unique manner, maximum depression occurred at 24 hours after poly rI:rC administration and the concurrent administration of inhibitors of protein synthesis (actinomycin D or cycloheximide) prevented this depression. These data suggest that poly rI:rC effects on the mixed function oxidases are not species specific although depression follows a time course shorter than that reported in the rat (maximum depression at 40 hours after poly rI:rC administration) and that depression occurs through the stimulation of a protein responsible for degrading cytochrome P-450.

Introduction. Several agents known for their ability to induce interferon have also been found to cause a marked depression of the cytochrome P-450 linked monooxygenase system (1,2,3). Although both induction of interferon and depression of the mixed function oxidases appear to be implicated in the response to viral infection, the relationship between the two is not clearly understood. It has been suggested that there is a causal relationship between the two events with interferon or some other component of the interferon system being responsible for depression of the MFO's (3,4). Since the MFO's are primarily responsible for the metabolism of drugs and other xenobiotics (5) their depression by inducers of interferon could influence the ability of an organism to rid itself of a variety of compounds.

The present study was undertaken (a) to determine the time course and extent of depression of cytochrome P-450 and several N-demethylase enzymes in

Abbreviations: Poly rI:rC, polyriboinosinic:polyribocytidylic acid. MFO, mixed function oxidase, DMN, N-nitrosodimethylamine.

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mice treated with the interferon inducer poly rI:rC and (b) to gain a better understanding of the mechanism by which this depression occurs. We hypothesize that poly rI:rC, in addition to stimulating interferon synthesis promotes the synthesis of protein(s) which depress the mixed function oxidases.

Methods. Poly rI:rC (m w > 100,000) obtained from Sigma Chemical Co. was administered to female ICR Swiss mice weighing 21-30 grams (100 μg/mouse, i.p.) at 12, 24, 48 and 72 hours prior to cervical dislocation. All mice were killed simultaneously to avoid circadian fluctuations in mixed function oxidase levels. Livers were removed and microsomes isolated by differential centrifugation. In order to determine whether there was a significant trend in the way that hepatic microsomal enzymes were affected by poly rI:rC, a one way ANOVA was performed on the difference between treatment and control values for each run (6). To test the effects of inhibitors of protein synthesis other groups of mice were administered either actinomycin D (6.25 μg/mouse, s.c.) or cycloheximide (30 mg/kg, s.c.) plus poly rI:rC at 24 hours prior to cervical dislocation. Groups of mice were also injected with either actinomycin D (12.5 g/mouse, s.c.) or cycloheximide (60 mg/kg, s.c.) plus phenobarbital sodium (60 mg/kg or 60 mg/mouse, i.p.) at 24 hours prior to cervical dislocation. Students t test was used to determine if actinomycin D or cycloheximide had significant effects when administered simultaneously with poly rI:rC (6).

Cytochrome P-450 content was determined by measuring the absorbance intensity of the carbon monoxide binding to sodium dithionite reduced microsomes (7). The activities of ethylmorphine N-demethylase and DMN-N demethylases I and II were determined by measuring formaldehyde production (8). Concentrations of ethylmorphine (10.0 mM) and DMN (2.0 mM and 50.0 mM for DMN N-demethylases I and II respectively) were incubated with microsomes (9) and a NADPH generating system at 37°C. DMN was incubated under yellow illumination to prevent light induced degradation and in a biohazard safety hood to insure safety of laboratory personnel.

Results. Figure 1 depicts the effects of a single dose of poly rI:rC on concentration of hepatic microsomal cytochrome P-450. Cytochrome P-450 content was maximally depressed to about 75% of control by 24 hours and had returned to control levels by 48 hours. Since there was no concurrent decrease in liver weight, depression of cytochrome P-450 is probably due to a selective effect of poly rI:rC on this group of enzymes.

The effects of poly rI:rC on the ethylmorphine and DMN N-demethylases are expressed in figures 2 and 3 respectively. Maximum depression of ethylmorphine N-demethylase to 56% of control occurred at 24 hours but unlike cytochrome P-450 some depression had occurred by 12 hours and control levels were not regained until 72 hours after treatment. DMN N-demethylase activities were maximally depressed to 80% of control at 24 hours, some depression of both enzymes had occurred by 12 hours and their activities were no longer significantly different from controls at 48 or 72 hours.

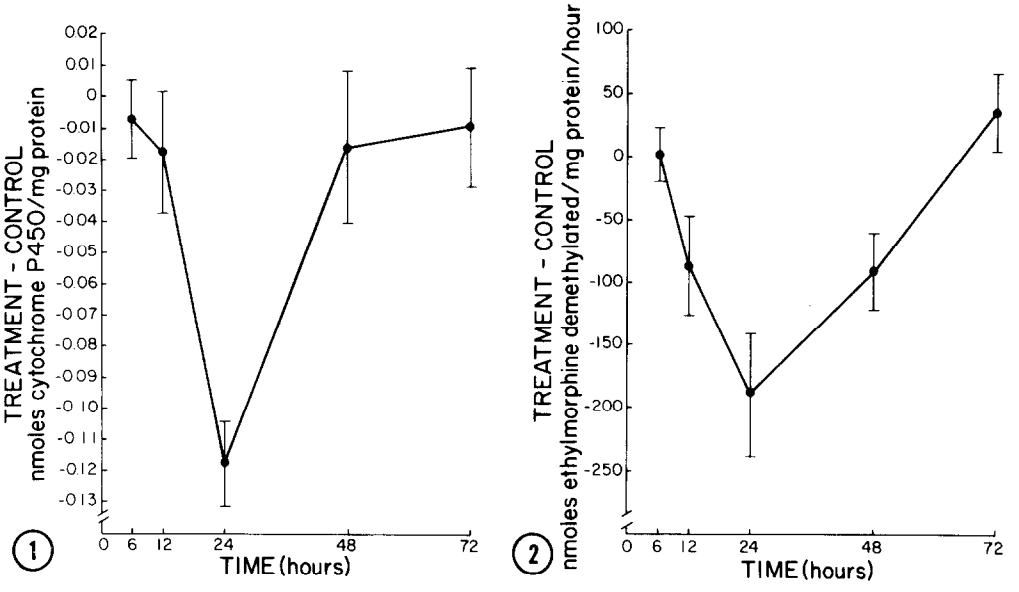

Figure 1. Time course of loss and recovery of cytochrome P-450 from hepatic microsomes following a single dose of poly rI:rC (100 μg/mouse, i.p.). Each point represents the mean ± S.E.M. of 10 determinations, each representing the difference between treated and control values derived from microsomes recovered from 4 livers. Trend was significant at $p < 0.01$.

Figure 2. Time course of loss and recovery of ethylmorphine N-demethylase activity of hepatic microsomes following a single dose of poly rI:rC (100 μg/mouse, i.p.). Each point represents the mean ± S.E.M. of treatment minus control values for 6 incubations, each incubation was done in duplicate and utilized microsomes from 4 livers. Substrate concentration was 10.0 mM. Trend was significant at $p<0.01$.

Actinomycin D prevented the depression of both cytochrome P-450 and the N-demethylases by poly rI:rC ($p < 0.01$). Cycloheximide also prevented the depression of cytochrome P-450 by poly rI:rC ($p < 0.01$). In addition actinomycin D and cycloheximide prevented the increase in liver cytochrome P-450 content caused by phenobarbital sodium ($p < 0.01$), a process which is known to require the synthesis of new protein (10). In the doses administered in these studies, neither cycloheximide nor actinomycin D had significant effects on mixed function oxidase levels when administered alone.

Discussion. A single dose of the interferon inducer poly rI:rC caused depression of cytochrome P-450 and ethylmorphine N-demethylase in mice. However the time course and extent of depression was different from that previously reported in rats (3). The fact that inducers of interferon cause

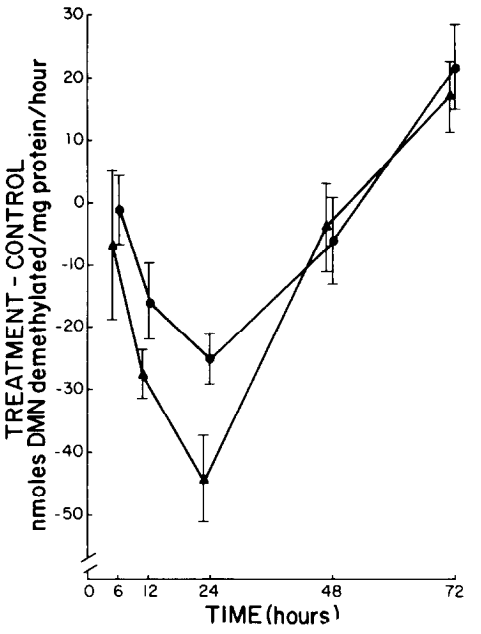

Figure 3. Time course of loss and recovery of DMN N-demethylase I (●-●) and DMN N-demethylase II (▲-▲) activities in hepatic microsomes following a single dose of poly rI:rC (100 μg/mouse, i.p.). Each point represents the mean ± S.E.M. of treatment minus control for 10 (DMN N-demethylase I) and 8 (DMN N-demethylase II) incubations, each incubation was done in duplicate and utilized microsomes from 4 livers. Substrate concentrations were 2.0 mM (DMN N-demethylase I) and 50.0 mM (DMN N-demethylase II). Trend was significant at $p<0.01$.

depression of the hepatic monooxygenase system in both rats and mice suggests that depression is not species specific and may therefore occur in man.

Cytochrome P-450, ethylmorphine N-demethylase and the DMN N-demethylases each responded to Poly rI:rC in a unique manner. Loss of activity of the N-demethylases paralleled the decrease in cytochrome P-450 content except at 12 hours where there was significant decrease in N-demethylase activity with no concurrent loss of cytochrome P-450. Ethylmorphine N-demethylase activity remained depressed at 48 hours despite the fact that cytochrome P-450 levels were back to those of control. It has been reported that poly rI:rC selectively depressed 3 out of 6 putative forms of cytochrome P-450 in rat liver (11). The results of this investigation indicate that poly rI:rC also selectively depresses specific cytochrome P-450 isozymes in mice.

Our findings together with those reported by other investigators (12,13) suggest a causal relationship between induction of interferon and depression of

the MFOs. In our study maximal depression of the mouse hepatic monooxygenase system occurred at 24 hours whereas maximal blood levels of interferon have been reported at 9-12 hours following a single dose of poly rI:rC in mice (12,13). Although depression of drug metabolism is a property of many interferon inducers (1,2,3) it is not clear whether this is a direct effect of interferon or is due to another common property of these agents. The temporal relationship between poly rI:rC administration, interferon induction and the resultant MFO depression supports the suggestion that interferon itself is the mediator responsible for decreased enzyme levels (14).

We found that depression of hepatic microsomal enzymes by poly rI:rC, requires the synthesis of new protein. Induction of interferon by poly rI:rC also requires synthesis of a variety of new proteins (15,16). Zerkle and Wade (17) demonstrated that poly rI:rC does not decrease ^{14}C Leucine incorporation into cytochrome P-450 in vivo. Therefore the proteins responsible for MFO depression are probably enzymes that either increase the rate of degradation of cytochrome P-450 by proteolysis or enhance the dissociation of the heme moiety from the apoenzyme.

Understanding the possible interactions between interferon, interferon inducers and drug metabolism is of great importance. Interferon is currently undergoing clinical trials as a potential therapeutic agent for the treatment of cancer and a variety of viral infections (18,19). Depression of the hepatic mixed function oxidases by interferon could affect the ability of individuals to metabolize xenobiotics, including activation of certain chemotherapeutic agents administered concurrently with interferon.

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